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Short communication

Metatranscriptome analysis of lactic acid bacteria during kimchi fermentation with genome-probing microarrays

Young-Do Nam [a,b], Ho-Won Chang [a,b], Kyoung-Ho Kim [a,b], Seong Woon Roh [a,b], Jin-Woo Bae [a,b,*]

[a] University of Science & Technology, KRIBB, Daejeon 305-806, Republic of Korea
[b] Department of Biology, Kyung Hee University, Seoul 130-701, Republic of Korea

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ABSTRACT

We constructed genome probing microarrays (GPM) that are specific to 39 lactic acid bacteria (LAB) in an effort to monitor microbial diversity and biological activity during the fermentation of kimchi, a traditional Korean vegetable product known to contain various health-promoting and immunity-boosting factors. Metagenomes and metatranscriptomes extracted from periodically sampled kimchi soup were labeled, hybridized and comparatively analyzed using GPMs. Each metatranscriptome was prepared by subtracting 16S rRNA and 23S rRNA from the total RNA, and selectively synthesizing mRNA-specific cDNAs from the rRNA-subtracted samples. Metagenomic analysis revealed 23 LAB related to kimchi fermentation [defined as bacteria with more than a 1% average relative composition (ARC)]. Metatranscriptome analysis revealed that, with the exception of two microorganisms, all LAB probed in the microarray contributed to kimchi fermentation. Moreover, the relative compositions of the major LAB remained unchanged (there was less than a 1.5% difference between the maximum and minimum values) in our metagenome analysis, while our metatranscriptome analysis revealed significant differences in the relative compositions of major LAB during fermentation (relative compositions changed by 2.4% to 9.5%). These data indicate that microorganisms that are less abundant in the flora (those with less than a 5% ARC in the metagenomic analysis) also participated in kimchi fermentation with relatively high activities.

1. Introduction

Kimchi is a fermented vegetable product made of various vegetables such as Chinese cabbage, radish, cucumber, and a seasoning mixture (red pepper powder, garlic, ginger, and green onion) (Kim et al., 2000). Kimchi contains various health-promoting components, including β-carotene, chlorophyll, vitamin C, and dietary fiber (Park et al., 1995). In addition, the anti-mutagen (Oh et al., 2005), anti-oxidation (Yoo et al., 2005) and angiotensin-converting enzyme inhibition (Yoo et al., 2004) activities of kimchi are thought to protect against disease. Bacteria isolated from kimchi produce beneficial enzymes, such as dextransucrase and alcohol/acetaldehyde dehydrogenase (Eom et al., 2007). Because of these beneficial properties, kimchi was nominated as one of the world's healthiest foods in a 2006 issue of *Health Magazine* (http://www.health.com/).

Kimchi is fermented by lactic acid bacteria (LAB); thus, a better understanding of the kimchi fermentation process can be obtained by studying the diversity of these bacteria. Various species of LAB involved in kimchi fermentation, including those in the *Leuconostoc* and *Lactobacillus* genera (Mheen and Kwon, 1984), have been identified using the conventional methods of isolation and phenotypic characterization (Lim et al., 1989; Park et al., 1990; Shin et al., 1996). Many LAB species have been identified using culture-dependent methods; however, the culture bias inherent in these methods makes it difficult to thoroughly identify the entire microbial community. Thus, recent studies have used molecular ecological methods to further characterize the microbial communities involved in kimchi fermentation. In particular, 16S rDNA cloning and amplified ribosomal DNA restriction analysis (ARDRA) (Kim and Chun, 2005), 16S rDNA amplification and denaturing gradient gel electrophoresis (DGGE) analysis (Lee et al., 2005) and genome probing microarrays (GPMs) (Bae et al., 2005) have been applied to microorganisms involved in kimchi fermentation.

DNA microarrays were first introduced in 1995 (Schena et al., 1995) and are typically constructed by arraying hundreds or thousands of DNA samples on small glass slides. Oligomer and cDNA microarrays differ with regard to the length of the spotted probes used. While specificity and sensitivity are critical prerequisites in any microarray experiment, cDNA microarrays generally lack specificity (Xu et al., 2001) and oligomer arrays generally lack sensitivity (Urakawa et al., 2002). Accordingly, oligomer microarrays are generally subject to enrichment or PCR amplification protocols to obtain a sufficient number of molecules (Revel et al., 2002). These processes alter the original environmental conditions, making it difficult to assess the size

* Corresponding author. University of Science & Technology, KRIBB, Daejeon 305-806, Republic of Korea. Tel.: +82 42 860 4628; fax: +82 42 860 4677.
E-mail address: baejw@kribb.re.kr (J.-W. Bae).

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of the microbial community and the quantity of a certain gene. In contrast to cDNA and oligomer microarrays, GPMs are highly specific and can distinguish microorganisms at the species level (microorganisms sharing 97% 16S rRNA sequence identity). In addition, GPMs boast superior sensitivity and are capable of detecting as little as 1 ng nucleic acid (Bae and Park, 2006; Bae et al., 2005; Chang et al., 2008b,c). However, GPMs are unable to elucidate the real dynamics of microbial communities during fermentation because these arrays use environmental DNA (metagenomes) as target samples, and DNA-based analyses reveal the total number of microorganisms rather than the biological activities of these organisms. Therefore, new technological approaches that use environmental mRNAs (metatranscriptomes) are better suited to investigate microbial dynamics during kimchi fermentation.

Here, we describe the construction of genome probing microarrays (GPM) specific to 39 LAB strains. The hybridization signal patterns of each metagenome and metatranscriptome sample were subsequently compared to determine the community structure and biological activity of bacteria during kimchi fermentation. To our knowledge, this is the first study to examine the microbial dynamics and biological activities of bacteria during kimchi fermentation using DNA- and mRNA-based microarrays.

2. Materials and methods

2.1. Kimchi sampling

We purchased 10 kg samples of kimchi from Chongga (http://www.chongga.com: this cite was lastly accessed at January 6th 2009), the most popular distributor in Korea. The kimchi was maintained at 4 °C, and 5 mL aliquots of kimchi soup were periodically obtained to measure the pH. Each sample was then stored at −80 °C in preparation for DNA and RNA extraction.

2.2. Bacterial strains and growth conditions

We used 39 strains of LAB as probes to assess microbial dynamics during kimchi fermentation (Table 1). The strains used in this study were obtained from the Korea Type Culture Collection (KCTC) and the German Collection of Microorganisms and Cell Cultures (DSMZ). Each strain was grown under the conditions recommended by the supplier. Cells were quickly harvested during exponential phase and frozen at −80 °C in preparation for DNA extraction.

Table 1
List of microorganisms probed in GPM

ID	KCTC no.	Strain name	Accession no.	ID	KCTC no.	Strain name	Accession no.
1	3108	*Lactobacillus plantarum*	X54259	21	3549	*Lactobacillus suebicus*	AJ575744
2	3161	*Lactobacillus amylophilus*	M58806	22	3594	*Lactobacillus reuteri*	X76328
3	3167	*Lactobacillus coryniformis* subsp. *coryniformis*	M58813	23	3597	*Lactobacillus amylovorus*	M58805
4	3498	*Lactobacillus brevis*	M58810	24	3606	*Lactobacillus agilis*	M58803
5	3501	*Lactobacillus animalis*	M58807	25	3608	*Lactobacillus vaccinostercus*	AJ417735
6	3542	*Lactobacillus graminis*	AM113778	26	3804	*Lactobacillus zeae*	D86516
7	3543	*Lactobacillus fructivorans*	M58818	27	3537	*Leuconostoc fallax*	AB023239
8	3546	*Lactobacillus murinus*	M58826	28	3525	*Leuconostoc carnosum*	X95977
9	3593	*Lactobacillus alimentarius*	M58804	29	3652	*Leuconostoc pseudomesenteroides*	X95979
10	3596	*Lactobacillus mali*	M58824	30	3544	*Leuconostoc fructosum*	X61140
11	3603	*Lactobacillus sakei* subsp. *sakei*	M58829	31	3528	*Leuconostoc lactis*	M23031
12	3611	*Lactobacillus kefiri*	AB024300	32	3530	*Leuconostoc mesenteroides* subsp. *dextranicum*	AB023246
13	3670	*Lactobacillus oris*	X94229	33	3504	*Weissella viridescens*	M23040
14	3681	*Lactobacillus farciminis*	M58817	34	3505	*Leuconostoc mesenteroides* subsp. *mesenteroides*	M23035
15	3767	*Lactobacillus curvatus*	AY204894	35	3773	*Leuconostoc argentinum*	AF175403
16	3802	*Lactobacillus sakei* subsp. *carnosus*	AY204892	36	3531	*Weissella paramesenteroides*	M23033
17	3814	*Lactobacillus versmoldensis*	AJ496791	37	3604	*Weissella minor*	M23039
18	13927	*Lactobacillus diolivorans*	AF264701	38	3499	*Weissella confusa*	AB023241
19	3112	*Lactobacillus fermentum*	M58819	39	3526	*Leuconostoc citreum*	AF111948
20	3503	*Lactobacillus parabuchneri*	AY026751				

2.3. DNA extraction and quantification

Genomic DNA from pure cultures and bulk community DNA from kimchi were isolated using a previously described bead-beating method (Yeates et al., 1998). All DNA samples were treated with RNase A (Sigma, St. Louis, MO) and analyzed on ethidium bromide-stained agarose gels stained prior to the fabrication and hybridization of microarrays. The extracted DNAs were further purified using the UltraClean® Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA) according to the manufacturer's recommendations, with the exception that the bead-beating step was excluded and the DNA solution was added to Solution MD1 instead of the MicroBead Solution. The concentrations of the purified DNA samples were determined in triplicate using a spectrophotometer (Nanodrop Technologies, Rockland, DE).

2.4. RNA extraction and SSU RNA subtraction

In preparation for RNA extraction, 5 mL aliquots of kimchi soup were pelleted by centrifugation at 5400 g for 10 min. The pellets were then lysed in 1 mL of Trizol reagent (Invitrogen Life Technologies, Karlsruhe, Germany) and 0.4 mL of zirconia-silica beads (0.1 mm diameter; Roth, Karlsruhe, Germany) in a Mini-BeadbeaterTM (Biospec Products, Bartlesville, USA). The RNA was then isolated according to the protocol established by Invitrogen. Contaminated DNA molecules were removed by incubating the samples with 50 U of DNase I (Fermentas) in the presence of 1.5 mM $MgCl_2$ and 80 U of RNaseOut (Invitrogen) at 37 °C for 30 min. The RNA was then precipitated by the addition of 5 M lithium chloride, washed with ethanol, and dried via vacuum centrifugation. After resuspending the RNA samples in 100 μl of DEPC-treated water, the concentration and purity of each sample was assessed in triplicate, using a spectrophotometer (Nanodrop Technologies, Rockland, DE). The 16S and 23S rRNAs were then removed using the MICROBExpress™ Bacterial mRNA Enrichment Kit (Ambion, Austin, TX). The total RNAs and rRNA-depleted RNAs were visualized on agarose gels stained with ethidium bromide.

2.5. Synthesis of cDNA from community mRNA

The 16S rRNA- and 23S rRNA-subtracted RNA samples were applied to the ExpressArt® Bacterial mRNA Amplification kit (Artus GmbH, Hamburg, Germany) to synthesize cDNA from the community mRNAs. Samples (5 μg each) were mixed with 3 μl of DEPC-treated

water, 1 μl of dNTP and 1 μl of primer TR. The cDNA synthesis mixture was incubated for 4 min at 65 °C and cooled at 37 °C. Subsequently, 4 μl of DEPC-treated water, 4 μl of 5X RT butter, 1 μl of RNase inhibitor and 1 μl of RT enzyme were added to the mixture. The first-strand cDNAs were synthesized during a 45 min incubation at 37 °C, followed by a 10 minute incubation at 45 °C, a 5 minute incubation at 50 °C and a 1 minute incubation at 37 °C. First-strand cDNA synthesis was terminated via incubation for 15 min at 80 °C and the remaining primers and RNA were removed by treatment with primer erase and RNase.

Second-strand cDNA synthesis was initiated with the addition of 10 μl DEPC-treated water, 3 μl of 5X extender buffer, 1 μl of primer B and 1 μl of a dNTP mixture. After a 1 minute incubation at 96 °C and a 1 minute incubation at 37 °C, 1 μl each of extender enzymes A and B was added to the mixture. At the conclusion of the reaction, the remaining primers were removed by the addition of 1 μl of primer erase. The newly synthesized double-stranded cDNA molecules were purified using the spin columns supplied with the kit.

2.6. Microarray construction

The genomic DNA extracted from each strain was diluted to a final concentration of 400 ng/μl in 0.1 X TE. Aliquots (5 μl) of each probe genome were transferred to a 384-well microplate and mixed with 5 μl of 2X microarray spotting solution (ArrayIt™, Telechem International, Inc., Sunnyvale, CA) in preparation for printing. The probes were arrayed onto Super-amine glass slides (25 mm by 75 mm; Telechem) at a spacing distance of 250 μm using a PixSys 5500 Printer (Cartesian Technologies, Inc. Irvine, CA) and four pins, in the presence of 55% to 58% relative humidity. Each probe set was printed in quadruplicate. The slides were cross-linked via exposure to 120 mJ of ultraviolet irradiation (UV Stratalinker 1800, Stratagene, La Jolla, CA). Immediately after blocking, the DNA samples were denatured by immersing the slides in deionized water (dH_2O) at 95 °C for 2 min. The microarrays were then rinsed briefly in 95% ethanol, air dried at room temperature and stored in a clean slide box at room temperature.

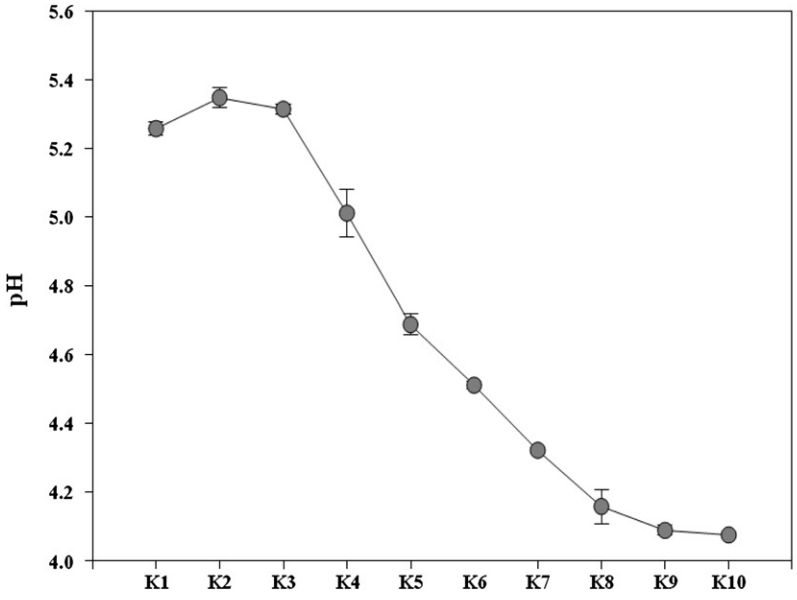
2.7. DNA and cDNA labeling

The bulk community DNAs and newly synthesized cDNAs were labeled using the BioPrime DNA Labeling System. The manufacturer's protocol was modified by mixing 15 μl of DNA (1 μg) with 20 μl of 2.5X random primers. The DNA was then denatured by boiling for 2 min, and was immediately chilled on ice. The denatured genomic DNA solution was then mixed with 15 μl of a labeling solution containing 5 mM each of dATP, dTTP, and dGTP; 2.5 mM dCTP; 2.5 mM Cy3-labeled dCTP (Amersham Pharmacia Biotech, Piscataway, NJ); and 40 U of Klenow fragment (Invitrogen, Carlsbad, CA). The reaction mixture was incubated at 37 °C for 3 h. The labeled target DNA was purified using QIAquick PCR purification column (Qiagen, Valencia, CA), concentrated in a Speedvac for 1 h, and resuspended in 4.35 μl of dH_2O in preparation for hybridization.

2.8. Microarray hybridization and data analysis

Microarray hybridizations were performed in triplicate (a total of 12 replicates per genomic DNA probe), unless otherwise noted, to enable statistical analyses. The hybridization solution consisted of 4.35 μl of labeled DNA, 8.75 μl of formamide (50% v/v), 3X SSC (1X SSC containing 150 mM NaCl and 15 mM trisodium citrate), 1.25 μg of unlabeled herring sperm DNA (Promega, Madison, WI), and 0.3% SDS in a total volume of 17.5 μl. Aliquots (7.5 μl) of hybridization mixture were deposited directly onto the slides and covered with a cover slip (i.e. 10 mm by 15 mm; Sigma). We then dispensed 15 μl of 3X SSC into the hydration wells on either side of the hybridization chambers (Corning). The microarray slides were placed into hybridization chambers, boiled for 5 min to denature the hybridization solution, and immediately plunged into a temperature-adjusted water bath, where they were allowed to hybridize overnight. After hybridization, each microarray slide was taken out of the water bath and the cover slip was immediately removed in wash solution 1 (containing 1X SSC and 0.2% SDS). The slides were washed for 5 min each in wash solution 1, wash solution 2 (0.1X SSC and 0.2% SDS), and wash solution 3 (0.1X SSC) at room temperature. The slides were then dried via centrifugation as described above.

A GenePix® 4000B microarray scanner (Axon instruments, Union City, CA) was used to scan the microarrays at a resolution of 10 μm. To ensure that all of the hybridized slides were scanned in a consistent manner, the laser power and photomultiplier tube (PMT) gain were adjusted to 100%. The scanned image displays were analyzed by quantifying the pixel density (intensity) of each hybridization spot using the GenePix® software program, version 6.0 (Axon



Phase	Total DNA (ng/ul)	Total RNA (ng/ul)
K3	36±5.4	34±10.1
K5	103±12.8	182±15.3
K8	180±7.7	209±18.3
K10	117±7.8	138±10.2

Fig. 1. Change in pH during kimchi fermentation. Kimchi soup was sampled during fermentation given the labels K1 to K10. Of these ten samples, K3, K5, K8 and K10 were used in the metagenome and metatranscriptome analyses. The concentrations of each nucleic acid are shown alongside the pH curve.

Instruments). The hybridization images presented in Fig. 3 are representative images that were automatically contrast-adjusted by the software. A grid of individual circles was superimposed onto the image to indicate the location of each quantified fluorescent DNA spot. Mean signal intensities were automatically determined for each spot. The local background signal was automatically subtracted from the hybridization signal of each individual spot. The signal-to-noise ratio (SNR) of each spot was calculated based on the formula (Zong et al., 2003): SNR = (signal intensity – background)/SD of background. In this formula, the 'background' measurement refers to the local spot background intensity. The 'SD of background' was calculated across all of the pixels using GenePix® software. Statistical analyses were performed using Microsoft Excel 2003 and Sigmaplot, version 8.0 (Jandel Scientific, San Rafael, CA). The relative SNRs were obtained by dividing the SNR values of each spot with the total mean SNR values of each microarray reaction. Then, average relative compositions (ARC) were acquired from 12 replicate data sets by dividing each relative SNR values with sum of relative SNR values.

3. Results and discussion

A previous study suggested that the use of GPM in conjunction with environmental RNA samples may yield a more precise indication of microbial diversity than other molecular methods (Bae and Park, 2006). However, as total RNAs are composed of more than 70% 16S and 23S rRNAs (Sung et al., 2003), cross-hybridization of 16S or 23S rRNA genes to phylogenetically similar targets might produce false-positive microarray signals when active microorganisms are observed in certain environments. Therefore, cDNA must be synthesized via the selective extraction of environmental mRNAs (metatranscriptomes) or via the subtraction of 16S rRNAs and 23S rRNAs to accurately monitor microbial activity and assess real microbial diversity. In this study, we constructed GPMs and investigated whether the combination of GPM technology and metatranscriptome hybridization could provide a more accurate assessment of microbial dynamics than GPM-environmental DNA sample analysis during the fermentation of kimchi.

The pH values of kimchi changed dramatically during the fermentation process. In this study, the pH of kimchi was 5.26 before fermentation, decreased to 4.07 after 12 days of fermentation at 4 °C, and remained stable for the rest of the fermentation period (Fig. 1). As the change in pH was mainly related to alterations in the diversity of LAB, 10 kimchi samples were collected along the pH gradient. Of these, four samples (from the initial, middle and final stages of fermentation) were hybridized to the microarray. Fig. 2A shows the bacterial RNA extracted from kimchi soup samples. The concentrations of DNA and RNA extracted from 5 mL aliquots of kimchi soup began to increase during phase 3 (K3) and slightly decreased at the end of fermentation (stage K10) (Fig. 1). To prevent the cross-hybridization of ribosomal RNA, RNA samples were purified via LiCl precipitation (Aalto et al., 2007) and 16S rRNAs and 23S rRNAs were subtracted using the MICROBExpress™ Bacterial mRNA Enrichment Kit (Ambion, Austin, TX). Fig. 2B reveals that almost all 16S rRNAs were subtracted; however, large quantities of 23S rRNAs were not removed at the end of the procedure. To specifically synthesize cDNA from mRNA, 16S rRNA- and 23S rRNA-subtracted RNAs were applied to the ExpressArt® Bacterial mRNA Amplification kit (Artus GmbH, Hamburg, Germany), which specifically synthesizes cDNA from bacterial mRNA.

The community DNA and newly synthesized cDNA samples (metatranscriptomes) were labeled with cy5-dUTP and cy-5 dCTP, respectively (Fig. 2C). The labeling pattern showed that the lengths of the community DNA were distributed in the 100–2000 base region, while the lengths of the cDNA were distributed in the 100–500 base region. This disparity in labeling patterns between the community DNA and cDNA samples may reflect a difference in the initial length of the nucleic acids (the community DNAs were more than 10 kb long, while the community RNAs were less than 3 kb long) (Fig. 2B). To compare the microbial diversity of the DNA and cDNA samples, the samples were hybridized and analyzed on the GPMs.

Traditionally, the fermentation of kimchi is processed by natural plant microflora and salted fish (jeotgal) (Chin et al., 2006). Subsequently, this food contains a diverse array of bacteria, archaea and yeast (Chang et al., 2008a). A few halophilic archaea were isolated from shrimp jeotgal (Roh et al., 2007a,b), a traditional fermented food and an important component of kimchi that is made from tiny shrimp and rock salt. Moreover, kimchi contains several other ingredients and fermentation can proceed under a variety of conditions; thus, the microorganisms involved in fermentation are very diverse and are always present in different proportions. Despite this microbial diversity, previous studies have established that certain bacterial genera (*Weissella*, *Leuconostoc* and *Lactobacillus*) play key roles in kimchi fermentation (Bae et al., 2005; Cho et al., 2006; Kim et al., 2008; Lee et al., 2005). Therefore, we constructed genome probing microarrays using 39 strains of LAB in the three genera named above (Table 1).

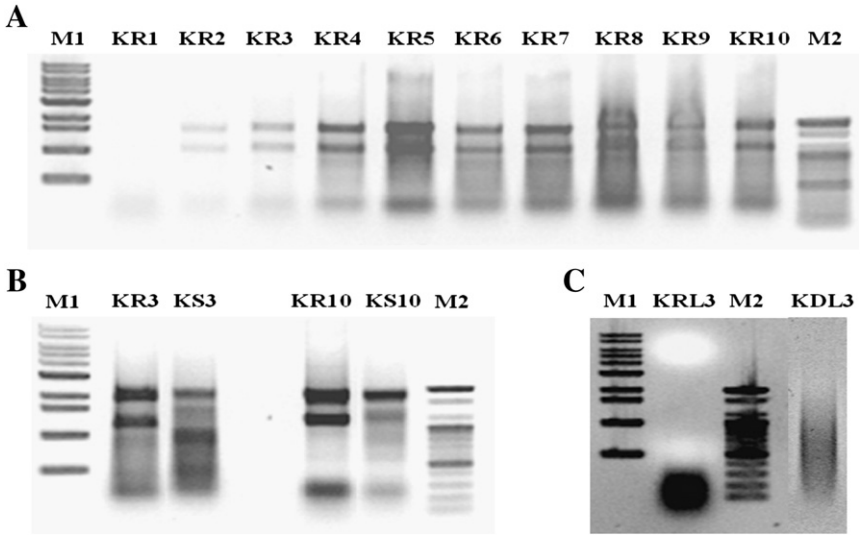


Fig. 2. All nucleotides were verified with electrophoretic analysis. (A) Community RNAs were periodically extracted from 10 kimchi samples (KR1–KR10). (B) The 16S rRNA and 23S rRNA were subtracted (KS3 and KS10) from community RNAs (KR3 and KR10). (C) The ribosomal rRNA subtracted RNA sample and community DNA were labeled with Cy-5 dUTP (KRL3 and KDL3, respectively). M1 and M2 denoted 1 kb and 100 kb markers, respectively (Bioneer, Deajeon, Korea).

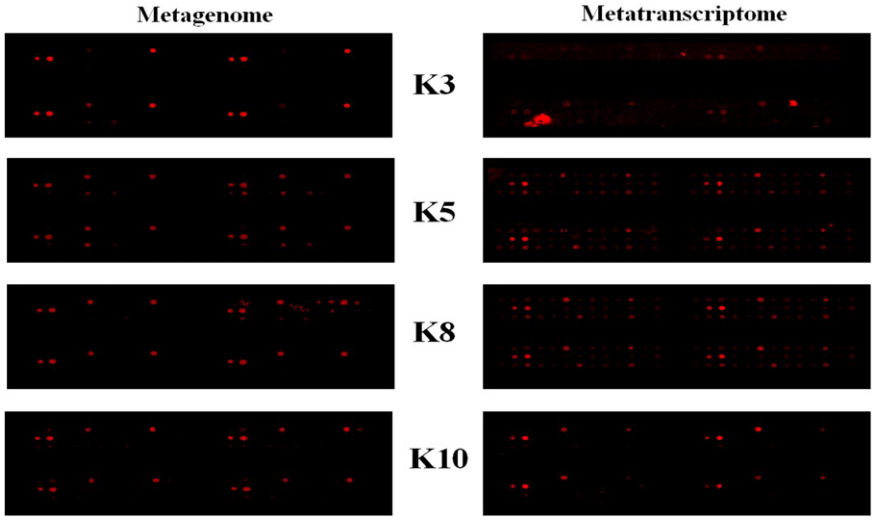


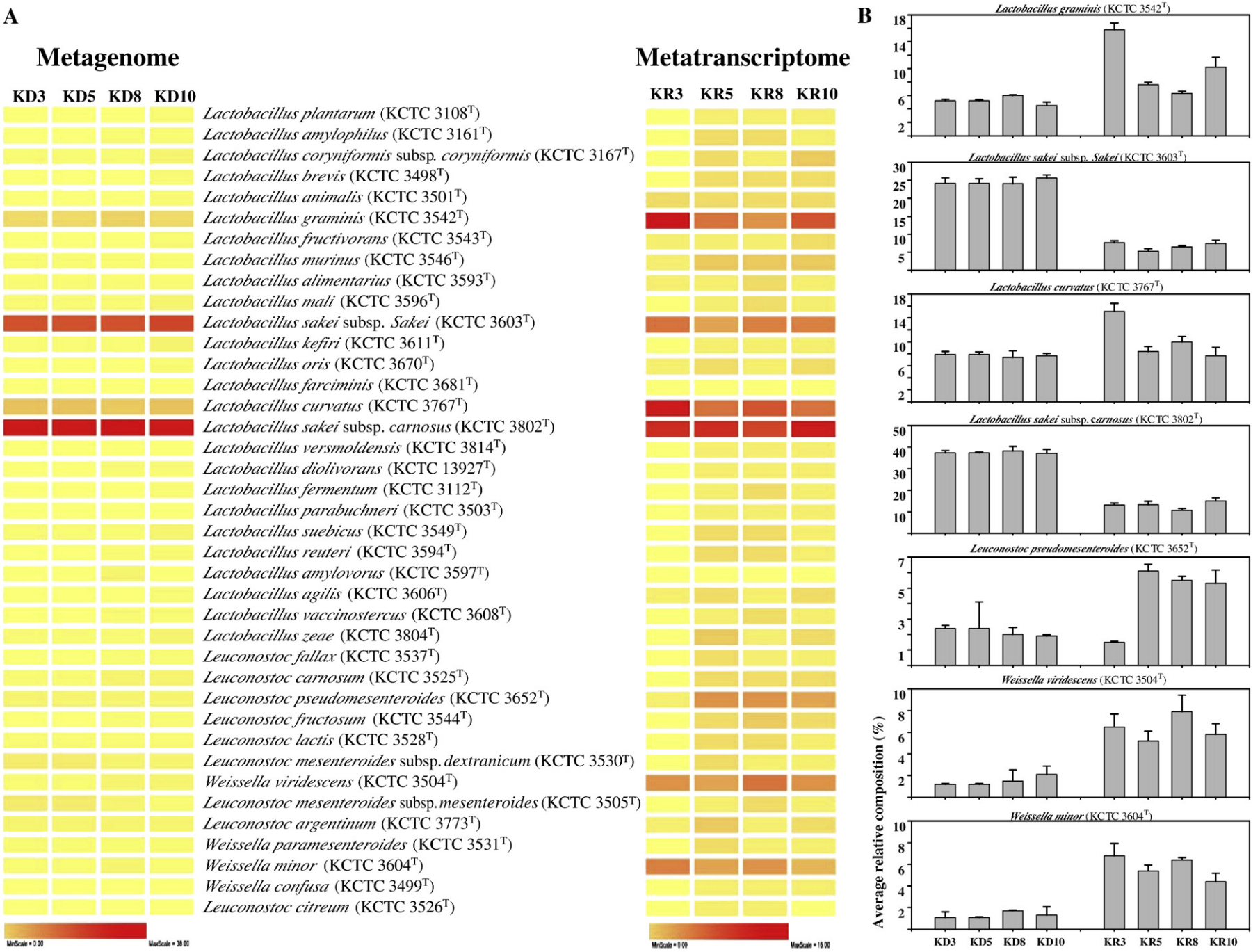
Fig. 3. Representative fluorescence images of DNA and RNA kimchi samples (i.e., samples K3, K5, K8 and K10) hybridized to the GPMs. The contrast of each image was automatically modulated using GenePix software to enable visualization with the naked eye.

Our metagenome analysis revealed that 23 of the 39 LAB species analyzed exhibited more than a 1% ARC (Fig. 4A). Previously, Bae et al. reported that *Lactobacillus* species (e.g., *Lactobacillus brevis*, *Lactobacillus alimentarius,* and *Lactobacillus oris*), *Leuconostoc* species (e.g., *Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides*), and some *Weissella* species were predominant in kimchi (Bae et al., 2005). However, our community metagenome analysis revealed only four major species of LAB (representing more than 5% of ARC) involved in kimchi fermentation. This may reflect differences in the number of probes, as only probes spotted on the GPMs could be detected. The four major species of bacteria detected in our study included *Lactobacillus sakei* subsp. *carnosus* KCTC 3802^T [37.4% average relative composition (ARC)], *Lactobacillus sakei* subsp. *sakei* KCTC 3603^T (24.5% ARC), *Lactobacillus curvatus* KCTC 3767^T (7.7% ARC) and *Lactobacillus graminis* KCTC 3542^T (5.2% ARC) (Figs. 3 and 4B). Previous studies revealed that *Lc. sakei* is a major component of the kimchi flora (Cho et al., 2006; Kim and Chun, 2005; Lee et al., 2005). *Lactobacillus curvatus* was also present in large numbers, although it was not identified as frequently as was *Lc. sakei*. While *Lc. graminis* exhibited 5.2% ARC during kimchi fermentation, this organism has never before been reported as a major component of the kimchi flora. Our DNA analysis revealed that the relative compositions of these microorganisms remained relatively stable during fermentation (the relative compositions changed by less than 1.5%).

In contrast to the results of our metagenome analysis, our metatranscriptome analysis revealed that 37 of 39 species exhibited ARC greater than 1%, and 7 species accounted for more than 5% of the relative composition (Fig. 4). Our metatranscriptome analysis revealed the four species identified via our metagenome analysis as well as three additional species of LAB, including *Leu. pseudomesenteroides* KCTC 3652^T (4.6% ARC), *Weissella viridescens* KCTC 3504^T (6.3% ARC) and *Weissella minor* KCTC 3604^T (5.7% ARC) (Fig. 4B). *Leuconostoc pseudomesenteroides* was previously identified as a dominant microorganism during kimchi fermentation (Bae et al., 2005). Likewise, *Weissella* species (e.g., *W. cibaria, W. confuse,* and *W. koreensis*) are also known mediators of kimchi fermentation (Bae et al., 2005; Cho et al., 2006; Kim and Chun, 2005; Lee et al., 2005). Our metatranscriptome analysis revealed that *Weissella* spp. may also contribute to kimchi fermentation. The relative compositions of the seven microorganisms identified in our RNA analysis fluctuated throughout the fermentation process (by 2.4% to 9.5%) (Fig. 4B).

The relative compositions of the seven species (those exhibiting more than 5% relative composition in the metagenome and metatranscriptome analyses) did not change during kimchi fermentation as shown by the metagenomic data (Fig. 4B). However, our metatranscriptome analysis revealed modulations in all of the major LAB species (as defined by a 4.75% mean max–min composition value). Specifically, *Lc. graminis* comprised 15.8%, 7.6%, 6.3% and 10.2% of the overall microbial flora as the fermentation process progressed, while *Lc. curvatus* comprised 15.1%, 8.4%, 10.0% and 7.7% of the microbial flora. This differs from our metagenomic data, which indicated that the relative composition of *Lc. graminis* and *Lc. curvatus* remained stable (5.2%, 5.2%, 6.0% and 4.5%; and 7.9%, 7.9%, 7.4% and 7.7%, respectively) during fermentation. The relative composition of *Leu. pseudomesenteroides* also appeared stable in the metagenome analysis (2.4%, 2.4%, 2.0% and 1.9%), but fluctuated in the metatranscriptome analysis (1.5%, 6.1%, 5.5% and 5.5%). While the sum of the ARC for the four most abundant species at each phase was over 69.7% in metagenome analysis, the sum of the ARC for the seven most abundant species at each phase was less than 56.8% in metatranscriptome analysis. These data indicate that metagenome analyses reveal the diversity of the microbial fauna but fail to describe the biological activity of the microorganisms, as metagenome analyses can detect live but inactive microorganisms. Moreover, the number of microorganisms comprising 2% to 5% of the microbial flora remained fairly stable in the metagenome analysis (3, 3, 4, and 4), but fluctuated in the metatranscriptome analysis (1, 10, 7 and 6). Thus, a combination of metagenome and metatranscriptome analyses is needed to accurately assess microbial dynamics. In our metagenome analysis, relatively minor populations of microorganisms remained undetected because the four most abundant microbial genomes were present in such large numbers. However, our metatranscriptome analysis revealed that less abundant microorganisms in metagenome analysis (those exhibiting less than 5% ARC) also participated in kimchi fermentation with relatively high activities.

Fig. 4. The quantities of LAB in each kimchi samples were detected with GPMs at a PMT gain of 700 V. Microarray hybridization patterns of the labeled DNAs (KD3, KD5, KD8, and KD10) and cDNAs (KR3, KR5, KR8, and KR10) from kimchi are shown in each column. (A) Each row represents the hybridization signal observed for each LAB when 1 μg of metagenome and metatranscriptome from the kimchi were used for hybridization. The SNRs were visualized by ArrayColor.exe (http://microarray.brc.re.kr), which produces more yellow squares from lower values of SNRs and more red squares from higher values. (B) While relative compositions of seven major microorganisms almost did not change during kimchi fermentation in metagenome analysis, relative composition of these microorganisms fluctuated in metatranscriptome analysis.


A
Metagenome
KD3 KD5 KD8 KD10
Metatranscriptome
KR3 KR5 KR8 KR10
Lactobacillus plantarum (KCTC 3108T)
Lactobacillus amylophilus (KCTC 3161T)
Lactobacillus coryniformis subsp. coryniformis (KCTC 3167T)
Lactobacillus brevis (KCTC 3498T)
Lactobacillus animalis (KCTC 3501T)
Lactobacillus graminis (KCTC 3542T)
Lactobacillus fructivorans (KCTC 3543T)
Lactobacillus murinus (KCTC 3546T)
Lactobacillus alimentarius (KCTC 3593T)
Lactobacillus mali (KCTC 3596T)
Lactobacillus sakei subsp. Sakei (KCTC 3603T)
Lactobacillus kefiri (KCTC 3611T)
Lactobacillus oris (KCTC 3670T)
Lactobacillus farciminis (KCTC 3681T)
Lactobacillus curvatus (KCTC 3767T)
Lactobacillus sakei subsp. carnosus (KCTC 3802T)
Lactobacillus versmoldensis (KCTC 3814T)
Lactobacillus diolivorans (KCTC 13927T)
Lactobacillus fermentum (KCTC 3112T)
Lactobacillus parabuchneri (KCTC 3503T)
Lactobacillus suebicus (KCTC 3549T)
Lactobacillus reuteri (KCTC 3594T)
Lactobacillus amylovorus (KCTC 3597T)
Lactobacillus agilis (KCTC 3606T)
Lactobacillus vaccinostercus (KCTC 3608T)
Lactobacillus zeae (KCTC 3804T)
Leuconostoc fallax (KCTC 3537T)
Leuconostoc carnosum (KCTC 3525T)
Leuconostoc pseudomesenteroides (KCTC 3652T)
Leuconostoc fructosum (KCTC 3544T)
Leuconostoc lactis (KCTC 3528T)
Leuconostoc mesenteroides subsp. dextranicum (KCTC 3530T)
Weissella viridescens (KCTC 3504T)
Leuconostoc mesenteroides subsp. mesenteroides (KCTC 3505T)
Leuconostoc argentinum (KCTC 3773T)
Weissella paramesenteroides (KCTC 3531T)
Weissella minor (KCTC 3604T)
Weissella confusa (KCTC 3499T)
Leuconostoc citreum (KCTC 3526T)
MinScale = 0.00
MaxScale = 38.00
MinScale = 0.00
MaxScale = 16.00
B
Lactobacillus graminis (KCTC 3542T)
Lactobacillus sakei subsp. Sakei (KCTC 3603T)
Lactobacillus curvatus (KCTC 3767T)
Lactobacillus sakei subsp. carnosus (KCTC 3802T)
Leuconostoc pseudomesenteroides (KCTC 3652T)
Weissella viridescens (KCTC 3504T)
Weissella minor (KCTC 3604T)
Average relative composition (%)
KD3 KD5 KD8 KD10 KR3 KR5 KR8 KR10

Most molecular methods of monitoring microbial diversity are based on metagenome analyses (Bae et al., 2005; Chin et al., 2006; Cho et al., 2006; Kim and Chun, 2005; Lee et al., 2005). However, these methods may not reveal the microorganisms responsible for kimchi fermentation because inactive bacteria (dormant cells, spores, and dead cells in which the DNA is not yet degraded) are detected as active cells (Bodrossy et al., 2006). In this study, we showed that metatranscriptome analyses can be used to monitor microbial dynamics during kimchi fermentation. Our results clearly demonstrate that the apparent composition of microbial communities can vary, depending on the use of DNA or mRNA samples. Furthermore, our metatranscriptome analysis revealed a more divergent microbial population and more accurately portrayed the changes in microbial activity. Thus, the methods and data presented in this study will contribute to the understanding of microbial population dynamics during the fermentation processes and improve manufacturing in a larger scale using artificially inoculated bacterial seed cultures.

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